EXHIBIT 606.D

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Postmortem Redistribution of Digoxin in Rats

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ABSTRACT: Adult male Wistar rats were treated with either 0.1 or 3 mg/kg body weight · day of digoxin for five days, then killed and stored at 4°C for 12 h in an attempt to mimic the normal preautopsy procedures in our hospital. In rats treated with 0.1 mg/kg body weight · day, the antemortem serum digoxin concentrations (SDC) were 1.1 ± 0.4 ng/mL while the 12-h postmortem concentration was markedly increased (16.3 ± 5.9 ng/mL) ($P < 0.01$). In rats treated with 3 mg/kg body weight · day, SDC was not changed significantly (11.2 ± 4.8 ng/mL antemortem and 13.3 ± 6 ng/mL postmortem). Postmortem redistribution of digoxin was assessed by injection of ^{125}I-labelled digoxin with or without pretreatment with the unlabelled drug. The results indicate that after death passive redistribution of digoxin may take place. When the SDC are within the therapeutic or low toxic range, digoxin may reenter the blood. High antemortem serum concentrations of digoxin may prevent such passive redistribution. Therefore, antemortem digoxin intoxication cannot be reliably inferred on the basis of high postmortem levels of the drug. Digoxin intoxication can be ruled out when postmortem SDC remain within the therapeutic range. The above changes cast doubt on some of the forensic and cardiologic literature, which has in the past been based on incorrect assumptions concerning postmortem behavior of digoxin.

KEYWORDS: pathology and biology, digoxin, blood, postmortem examinations, pharmacokinetics, redistribution

Digitalis intoxication is a serious clinical emergency that, in adults, has been reported to be associated with digoxin serum concentrations higher than 2.5 ng/mL [*1*]. Since the drug is frequently administered to critically ill patients, the possibility of digitalis intoxication must be considered in every unexplained death of a digitalized patient [*2*]. Recently, several studies have reported postmortem serum digoxin concentrations significantly higher than those normally measured during life [*3–5*]. Holt [*6*] and Doherty [*7*] have suggested that after death a new equilibrium between the blood and tissues is established, resulting in a higher digoxin concentration in the blood. However, no controlled experiment has been reported to prove this assumption.

The phenomenon does create difficulties in interpretation of postmortem serum digoxin levels in cases where antemortem serum levels are not available. Moreover, studies in which postmortem tissue versus plasma concentrations of digoxin have been assessed are further confounded since it is possible that these values may not reflect the normal distribution of the drug in life, but rather a new and radically altered distribution [*5,8–10*]. There are no studies of changing digoxin distribution in the terminal stages of either acute or chronic cardiac failure. The available data imply that substantial shifts in distribution may occur.

It was the aim of our studies to describe any discrepancies that may exist between antemortem and postmortem digoxin levels in the blood and in various tissues using both thera-

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peutic and toxic digoxin levels in a rat model. In the second stage of this experiment we studied possible postmortem redistribution of the drug using radiolabelled digoxin.

Materials and Methods

Antemortem and Postmortem Digoxin Serum Levels

Sixteen adult male Wistar rats were treated with either 0.1 (eight rats) or 3 mg/kg body weight (eight rats) of intramuscular digoxin per day for five days. These dose regimens were chosen because of the high LD_{50} of digoxin in the rat, which exceeds by far the human values [11] and the rapid elimination rate of the cardiac glycoside in rodents. On the sixth day they were killed by cervical dislocation and serum samples for measurement of digoxin levels were obtained immediately from the heart. Carcasses were then stored in a refrigerator at 4°C for 12 h, in an attempt to mimic the normal preautopsy procedures in our hospital. After this storage period samples for measurement of digoxin concentrations were again obtained from the heart. Digoxin serum levels were assessed by the routine radioimmunoassay (New England Nuclear Ltd.).

Postmortem Redistribution of Digoxin

Five adult male Wistar rats were injected intramuscularly with ^{125}I-labelled digoxin (New England Nuclear) 0.015 μCi with specific activity of 2000 dpm/pg. Two hours later they were killed by cervical dislocation and samples of cardiac muscle, diaphragm, liver, and kidney were removed. Renal cortical, liver, heart, and diaphragm radioactivity was measured in a γ counter (dpm per gram of wet tissue) and compared to the blood radioactivity (per gram of blood). The carcasses of these five rats were then stored as described above in a refrigerator at 4°C for 12 h, following which various tissue samples were again removed, radioactivity reassessed and compared to blood radioactivity.

In the above studies comparisons were made by the two-tailed student's *t* test for unpaired results.

In a further study of postmortem digoxin redistribution five adult male Wistar rats were treated for five days with unlabelled digoxin 1 mg/kg body weight. On the sixth day they were injected with ^{125}I-labelled digoxin 0.015 μCi, 2 h after the daily injection of the unlabelled drug. Two hours later they were killed and samples of cardiac muscle, diaphragm, liver, and kidney were removed. Renal cortical, liver, heart, and diaphragm radioactivity was measured (dpm per gram of wet tissue) and compared to blood radioactivity (per gram of blood). As in earlier experiments, the carcasses were subsequently maintained in a refrigerator at 4°C for 12 h, following which the radioactivity of the various tissues was compared to the serum reactivity.

Results are expressed throughout the text as mean ± standard deviation. Results from simultaneous studies were compared by the two-tailed student's *t* test for paired results.

Results

The mean digoxin concentration of serum obtained from heart of rats treated with digoxin dose of 0.1 mg/kg body weight was within the therapeutic range for humans (1.1 ± 0.4 ng/mL), while the mean 12-h postmortem concentration was markedly increased (16.3 ± 5.9 ng/mL) ($P < 0.01$).

In the group of rats treated with a high digoxin dosage (3 mg/kg body weight) the antemortem level of serum digoxin was within the toxic range for humans (11.2 ± 4.8 ng/mL). In this group the mean serum concentration although slightly increased did not change significantly 12 h after death (13.3 ± 6 ng/mL).

Tissue : Plasma Distribution

Animals Injected with Radiolabelled Digoxin—The tissue : blood distribution ratio of ^{125}I digoxin is shown in Table 1. The antemortem data indicate high tissue : blood ratio of digoxin in the kidney, liver, diaphragm, and cardiac muscle.

In the 12-h postmortem specimens, the concentration of the labelled digoxin in the blood was much higher than found in the antemortem samples (960 and 155 cpm/g, respectively, $P < 0.001$). Primarily because of this increase in blood digoxin concentration, tissue : blood ratios for labelled digoxin significantly decreased to approach unity in all tissues examined.

Previously Digitalized Animals Injected with Radiolabelled Digoxin—The tissue : blood distribution ratio of ^{125}I digoxin in animals given radioactive digoxin after earlier digitalization is shown in Table 2. The antemortem data demonstrate low tissue : blood ratios in the various tissues studied. These ratios are significantly lower than those observed in undigitalized rats receiving a single injection with radiolabelled digoxin ($P < 0.05$). Twelve hours later the tissue : blood ratio of labelled digoxin was found to be unchanged in the digitalized rats in all tissues tested.

Discussion

In common with earlier reported human studies [3–5], the first part of our experiment indicates that in the rat low antemortem serum levels during life tend to increase significantly after death. On the other hand, this phenomenon was not observed following exposure of test animals to higher digoxin dosage. In that situation the postmortem levels were similar to the higher antemortem concentrations. The combination of these two observations leads to the suggestion that passive redistribution of digoxin may occur after death. During life it appears that most of the drug is actively accumulated by cardiac and skeletal muscle as well as by kidney and liver [12]. The tissue : serum digoxin ratio during life is high above unity for these tissues, accounting for the large distribution volume of the drug [12]. Spiehler has found high concentration of digoxin in the brain of toxic cases and not of therapeutic

TABLE 1—*Antemortem and 12-h postmortem tissue : blood distribution ratio of ^{125}I digoxin in undigitalized rats injected with the radiolabelled digoxin 2 h before being killed.*

Ratio	Antemortem	12-h Postmortem	Significance of Change
Kidney : blood	7.9 ± 5.4	1.1 ± 0.5	$P < 0.05$
Liver : blood	8.8 ± 2.3	1.2 ± 0.3	$P < 0.01$
Cardiac : blood	10.6 ± 6.6	0.9 ± 0.2	$P < 0.05$
Diaphragm : blood	6.1 ± 1.3	0.8 ± 0.2	$P < 0.05$

TABLE 2—*Antemortem and 12-h postmortem tissue : blood distribution ratio of ^{125}I digoxin in rats exposed for five days to toxic doses of the drug.*

Ratio	Antemortem	12-h Postmortem	Significance of Change
Kidney : blood	2.4 ± 0.2	2.3 ± 0.3	N.S.[a]
Liver : blood	0.9 ± 0.2	0.9 ± 0.2	N.S.
Cardiac : blood	0.9 ± 0.2	0.9 ± 0.3	N.S.
Diaphragm : blood	1.0 ± 0.2	1.6 ± 0.6	N.S.

[a] N.S. = No significance.

cases, thus suggesting that digoxin content of the medulla may be useful in confirmation of antemortem blood digoxin concentrations [*13*]. After death, it appears that cessation of the active modulating accumulation process takes place, and, as a result, digoxin is redistributed passively from tissues containing digoxin in high concentration into areas of lower concentrations such as the blood. On the other hand, when serum concentrations of digoxin are extremely high because of acute intoxication the lack of a gradient may block redistribution.

To study empirically this hypothesis, we monitored postmortem digoxin redistribution using digoxin labelled with ^{125}I. We measured the tissue : blood ratios for various tissues at the time of death and 12 h later. Our results indicate that in undigitalized rats given an acute dose of digoxin, digoxin accumulates during life in the various tissues in concentrations much higher than the serum concentrations. These results are consistent with DiGregorio's observations on the tissue distribution of digoxin in the rat [*12*], as well as with human studies [*5,8–10*].

The tissue : blood concentration ratio for digoxin 12 h after death approaches unity, indicating that in the various tissues equilibrium of digoxin concentrations with blood concentrations has been achieved. This indicates that after death the drug tends to leave the cells and to enter the extracellular as well as the intravascular compartment.

Conversely, redistribution of digoxin was inhibited in animals previously exposed to pretreatment with toxic doses of the drug in nonlabelled form. The radiolabelled digoxin given after such pretreatment did not enter tissues in large quantities in these animals probably because of earlier saturation of digoxin binding sites by the excessive amounts of unlabelled digoxin. During the 12 h after death a redistribution of digoxin did not take place as a result of the relative balance between the organ : blood distribution already established in the digitalized animals.

Our findings have several implications for the interpretation of postmortem digoxin levels in serum as well as in various tissue.

1. After death, passive redistribution of digoxin may take place. When the serum concentrations are within the therapeutic or low toxic range it appears likely that digoxin will reenter the blood. High antemortem serum concentrations of digoxin may prevent such a passive redistribution.

2. Antemortem digoxin intoxication cannot be reliably inferred on the basis of high postmortem levels of the drug alone.

3. Digoxin intoxication can be ruled out when postmortem serum concentrations remain within the therapeutic range.

4. Since the redistribution of digoxin depends upon the time after death, and probably on other, as yet unknown factors, any extrapolation from postmortem data to the distribution of the drug in life may be tenuous. The changes reported above cast doubt on some of the cardiologic literature [*10–11,14*], which have reported postmortem tissue digoxin concentrations as if these values accurately represent the antemortem distribution of the drug.

There is a pressing need for better postmortem human studies of digoxin distribution for purposes of both medicolegal and clinical understanding.

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